SECTION HEAD

007188

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 18 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

PC 059101

SUBJECT: CHLORPYRIFOS: 4-Day Dermal Probe and 21-Day Dermal Toxicity Studies in Fischer 344 Rats - Identification No. 464-404; Record No. 239190; MRID No. 40972801; HED Project No. 9-0759; Caswell No. 219AA.

FROM: Alan C. Levy, Ph. D. Alan C. Levy 5-16-89
Toxicologist, Review Section I
Herbicide/Fungicide/Antimicrbial Support Branch (II)
Health Effects Division (HED), H7509C

TO: Dennis Edwards PM 12
Registration Division (H7505C)

THROUGH: Yiannakis M. Ioannou, Ph. D. Y.M. Ioannou 5-17-89
Acting Section Head, Review Section I
HFAS Toxicology Branch (II), HED (H7509C)

and

Marcia van Gemert, Ph. D. M van Gemert 5/17/89
Acting Branch Chief, HFAS Toxicology Branch (II)
HED (H7509C)

Registrant: Dow Chemical U.S.A.

Action Requested: Review 4-day dermal probe and 21-day dermal toxicity studies in Fischer 344 rats treated with CHLORPYRIFOS.

Results

The studies were reviewed by Dynamac Corporation. A copy of the Data Evaluation Record (DER) is attached.

4-Day Probe Study: This study was conducted at dermal doses of 0, 1, 10, 100 or 500 mg CHLORPYRIFOS (in corn oil)/kg/day. There were marked dose-related decreases in plasma and RBC cholinesterase levels at the three highest dose levels. There were no apparent treatment related effects on the skin or on any other parameter examined.

21-Day Study: This study was conducted at dermal doses of 0, 0.1, 0.5, 1 or 5 mg CHLORPYRIFOS (in corn oil)/kg/day (5 days/week for a total of 15 applications of 6 hours each). No signs of toxicity (including cholinesterase inhibition) were observed at any dose.

Conclusion

As no signs of systemic or dermal toxicity were observed at any doses in the 21-day rat study, the following are the effect levels:

Toxicity No Observed Effect Level (NOEL) = 5 mg/kg/day (HDT)
Toxicity Lowest Observed Effect Level (LOEL) = 10 mg/kg/day
(based on plasma and RBC cholinesterase inhibition in the 4-day study)

Even though there was no toxic effect observed at doses up to and including 5 mg/kg/day in the 21-day study, the "toxic effects" aspect (as required by the Guidelines) is considered to be fulfilled due to the cholinesterase inhibition observed at 10 mg/kg/day in the 4-day dermal probe study.

This 21-day study is classified Core Supplementary. It may be upgraded if the Registrant's response to the following is acceptable:

The Registrant is requested to provide data concerning the results of homogeneity, concentration and stability of the test article dosing solutions.

Tox Chem No 219AA (CHLORPYRIFOS) File Int Jl 86 Comment Date

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
DERMAL: 4-DAY PROBE 21-DAY STUDY SPECIES: RAT DOW CHEMICAL U.S.A. 9/1/88	CHLORPYRIFOS 100%	MRID 40972801	FISCHER 344 RATS 4-DAY: 0, 1, 10, 100 & 500 mg/kg/day 21-DAY: 0, 0.1, 0.5, 1 & 5 mg/kg/day RESULTS 4-DAY: DECREASED PLASMA & RBC CHOLINESTERASE AT 10, 100 & 500 mg/kg/day NO DERMAL OR SYSTEMIC EFFECTS 21-DAY: NO DERMAL, SYSTEMIC OR CHOLINESTERASE EFFECTS NOEL = 5 mg/kg/day (HDT) LOEL = 10 mg/kg/day (BASED ON PLASMA AND RBC CHOLINESTERASE INHIBITION IN THE 4-DAY DERMAL PROBE STUDY)		SUPPLEMENTARY (DATA REQUESTED FOR HOMOGENEITY, CONCENTRATION AND STABILITY)

FINAL

7188

EPA No.: 68D80056
DYNAMAC No." 168-A
TASK No.: 1-68A
April 6, 1989

DATA EVALUATION RECORD

CHLORPYRIFOS

21-Day Dermal Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

EPA No. 68D80056
DYNAMAC No.: 168-A
TASK No.: 1-68A
April 6, 1989

DATA EVALUATION RECORD

CHLORPYRIFOS

21-Day Dermal Toxicity Study in Rats

REVIEWED BY:

Margaret E. Brower, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: _____
Date: _____

William McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: _____
Date: _____

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____
Date: _____

Alan C. Levy, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H7509C)

Signature: Alan C. Levy
Date: 5-16-89

Mike Ioannou, Ph.D.
EPA Acting Section Head,
Section I
Toxicology Branch II (H7509C)

Signature: M. Ioannou
Date: 5/16/89

DATA EVALUATION RECORD

GUIDELINE § 82-2

STUDY TYPE: 21-Day Dermal Toxicity Study in rats.

MRID NUMBER: 409728-01

TEST MATERIAL: Chlorpyrifos

SYNONYM(S): Dursban, Lorsban, Brodan, o,o-diethyl-o-(3.5.6-trichloro-2-pyridinyl)phosphorothioate.

STUDY NUMBERS; K-044793-085, K-044793-086

SPONSOR: The Dow Chemical Company, Midland, MI.

TESTING FACILITY: Mammalian and Environmental Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI.

TITLE OF REPORT: Chlorpyrifos: 4-Day Dermal Probe and 21-Day Dermal Toxicity Study in Fischer 344 Rats.

AUTHOR(S): Calhoun, L.L, and Johnson, K.A.

REPORT ISSUED: September 1, 1988.

CONCLUSIONS: Under the conditions of the study, no systemic or dermal compound-related signs of toxicity were found to occur after repeated dermal exposure to chlorpyrifos at dose levels of 0, 0.1, 0.5, 1, or 5 mg/kg/day for 21 days. The NOEL for the 21-day study is 5 mg/kg/day, the highest dose tested. Dose-related decreases in plasma and erythrocyte cholinesterase activity were exhibited at 10, 100, and 500 mg/kg/day in a 4-day dermal probe study, which was conducted to determine dosage levels for the 21-day study.

Classification: Core Supplementary.

A. MATERIALS:

1. Test Compound: Chlorpyrifos; Description: white granular crystal; Lot No.: AGR 219646: Purity: 100 ± 0.1%

2. Test Animals: Species: rat; Strain: Fischer 344; Age: 12 weeks at study initiation; Weight: males--265.4 to 291.7 g, females--171.1 to 192.7 g; Source: Charles River Breeding Laboratories, Inc., Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Following 7 days of acclimation, animals were assigned to the following test groups using a stratified body weight randomization procedure:

Test group	Dose level (mg/kg/day)	Main Study (21 days) Males	Females
1 Control	0	5	5
2 Low (LTD)	0.1	5	5
3 Mid (MDT) A	0.5	5	5
4 MID (MDT) B	1	5	5
5 High (HDT)	5	5	5

Animals were housed individually in an environmentally controlled room with a 12-hour light/dark cycle.

Rationale for Dose Selection: The dose levels and conditions for this 21-day study were based on a 4-day probe study in which groups of four female Fischer 344 rats were treated via dermal application at dose levels of 0, 1, 10, 100, or 500 mg chlorpyrifos (in corn oil)/kg/day for 4 consecutive days. Slight erythema was exhibited in two of four females receiving 1 or 10 mg/kg/day; no other signs of skin irritation were exhibited. Plasma and erythrocyte cholinesterase activities were markedly decreased in a dose-related fashion in females receiving 10, 100, or 500 mg/kg/day (54.8, 8.6, and 2.5% of the concurrent control plasma cholinesterase activities, respectively, and 83.8, 50.7, and 25.4% of the concurrent control erythrocyte cholinesterases activities, respectively.) Statistical analyses were not conducted. Cholinesterase activities of females receiving 1 mg/kg/day were slightly decreased (numerically only) when compared with concurrent controls; the study authors reported these values to be within the range of historical controls. No other compound-related signs of toxicity were exhibited. Based on these results, dose levels for the 21-day study were 0, 0.1, 0.5, 1, and 5 mg chlorpyrifos/kg/day.

Dose Preparation: Stability concentration and homogeneity of chlorpyrifos in corn oil were determined during the 4-day probe study. [1,2,3,4] The study authors reported the dose solutions to be homogeneous and stable for up to 30 days. Recovery concentrations of the diet dosages were reported to be within 88 to 99% of target for the probe study and 79 to 100% for the 21-day study. Topical dosages of 0, 0.1, 0.5, 1, or 5 mg chlorpyrifos (in corn oil)/kg/day were applied at an equivalent dosing volume (not reported). Analytical results of these analyses were not included in this study report.

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[1]Payne, R.D. (1987) Analytical Data Sheet (HET-K-044793-085-2. Analytical Report of the Dow Chemical Company, Midland, MI.

[2]Kastl, P.E. (1988) Analytical Data Sheet (HET-K-044793-85-3). Analytical Report of the Dow Chemical Company, Midland, MI.

[3]Freshour, N. L. (1987) Analytical Data Sheet (HET-K-044793-085-1). Analytical Report of the Dow Chemical Company, Midland, MI.

[4]Wall, A. J. (1987). Analytical Data Sheet (HET-K-044793-086-1). Analytical Report of the Dow Chemical Company, Midland, MI.

Preparation of Animal Skin: Prior to dosing, the hair was removed from the back of each animal (approximately 12 cm^2) by clipping. Animals were reclipped when necessary throughout the study period. The test material was applied to the clipped area and uniformly spread over the application site once per day, 5 days/week, for a total of 15 applications in 21 days. The area was covered and wrapped with an occlusive bandage for 6 hours, after which time the test site was wiped with a moistened towel. Control animals were treated with the vehicle control formulation (without active ingredient) in volumes equivalent to the test animals.

3. Food and Water Consumption: Animals received food (certified Laboratory Chow #5002) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, hematology and clinical chemistry data, cholinesterase activity, urinary specific gravity, and organ weights were analyzed using Bartlett's test for equality of variances followed by analysis of variance and Dunnett's test. White blood cell differential counts and food consumption were analyzed using descriptive statistics. Statistical outliers were identified using a sequential outlier test.

5. Quality Assurance: A quality assurance statement was signed and dated August 10, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected once daily for signs of morbidity and mortality. Dermal evaluations were conducted daily. A functional observational battery was conducted on all animals prior to necropsy. This included the rating of evasive and locomotive behavior and responsiveness to touch and pain. Muscle tone, tremor, hair coat condition, salivation, lacrimation, and urine and fecal staining also were observed.

   Results: It was reported that there were no compound-related signs of systemic toxicity; a reddish-brown exudate was exhibited around the eyes of dosed and control males and females. There was no indication of skin irritation in dosed animals. There were no mortalities. Results of the functional observational battery were similar in dosed and control males and females.

2. Body Weight: Rats were weighed prior to study initiation and weekly thereafter.

Results: Body weights of dosed and control males and females were found to decrease slightly (2 to 4%) during study week 1; however, dosed and control animals recovered lost weight during study week 2 and continued to gain weight until study termination (Table 1). Body weights were similar between dosed and control males and females. The initial weight loss was considered to be the result of stress associated with increased handling.

2. Food Consumption and Compound Intake: Consumption was determined, and mean daily diet consumption was calculated weekly.

Results: There were no compound-related changes in food consumption during the study period.

4. Ophthalmological examinations: Ophthalmological examinations were performed prior to study initiation.

Results: Results of ophthalmological examinations were not reported.

5. Hematology an Clinical Chemistry: Blood was collected from the orbital sinus prior to necropsy for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*		Mean corpuscular HBG concentration (MCHC)
X	Erythrocyte count* (RBC)		Mean corpuscular volume (MCV)
X	Platelet count*		Coagulation:thromboplastin time (PT)
	Reticulocyte count (RETIC)*		
	Red cell morphology		

Results: There were no toxicologically important effects on hematologic parameters. All group mean values were similar and within the normally expected range.

* Recommended by Subdivision F (October 1982) Guidelines.

TABLE 1. Representative Results of Mean Body Weight (± S.D.) of Rats Treated Dermally with Chlorpyrifos for 21 Days[a]

Dosage level (mg/kg/day)	Mean body weights (g) at day pretest	14	21
		Males	
0	277.5 ± 9.6	270.9 ± 6.9	289.7 ± 5.0	299.3 ± 3.9
0.1	278.0 ± 7.5	270.9 ± 7.4	285.1 ± 8.7	295.6 ± 9.3
0.5	278.1 ± 5.8	265.9 ± 4.7	281.3 ± 4.6	289.4 ± 6.0
1	279.8 ± 9.9	273.4 ± 9.4	287.5 ± 9.0	294.5 ± 11.8
5	297.3 ± 7.5	269.3 ± 7.5	286.2 ± 6.4	293.2 ± 10.0
		Females	
0	177.8 ± 6.4	169.9 ± 7.5	180.8 ± 6.8	185.3 ± 8.6
0.1	178.9 ± 6.4	170.9 ± 6.9	183.6 ± 7.1	186.8 ± 6.2
0.5	180.6 ± 8.2	173.5 ± 9.2	185.4 ± 8.8	191.5 ± 8.8
1	181.2 ± 8.5	173.8 ± 9.3	184.7 ± 9.3	188.3 ± 12.7
5	181.2 ± 7.2	174.1 ± 7.2	182.2 ± 6.4	186.1 ± 5.8

[a]Based on five animals/sex/dose.

Data was extracted from Tables 12 and 13, pages 34 and 35 of the Report.

b. Clinical Chemistry

	Electrolytes
X	Calcium+
X	Chloride+
	Magnesium+
X	Phosphorus+
X	Potassium+
X	Sodium+

	Enzymes
X	Alkaline phosphatase (ALP)
X	Cholinesterase (plasma, RBC, brain)
	Creatinine phosphokinase+
	Lactic acid dehydrogenase
X	Serum alanine (SGPT)+
X	Serum aspartate aminotransferase (SGOT)+
	Gamma glutamyltransferase (GGT)

	Other
X	Albumin+
	Albumin/globulin ratio
X	Blood creatinine+
X	Blood urea nitrogen+
X	Cholesterol+
x	Globulins
X	Glucose+
	Total bilirubin+
	Direct bilirubin
X	Total protein+
X	Triglycerides

Results: There were no toxicologically important effects on clinical chemistry parameters. Cholinesterase activity results are presented in Table 2. The erythrocyte cholinesterase activity of males dosed with 5 mg chlorpyrifos/kg was slightly decreased when compared with concurrent controls; however, this decrease was found to be the result of one outlier (animal No. 87A7140) with an erythrocyte cholinesterase activity of 8.9 U/mL. If this statistical outlier were omitted in calculating the mean RBC cholinesterase activity, the value would be similar to that of controls. The plasma cholinesterase activity of high-dose females was found to be slightly decreased when compared with concurrent controls; however, this value was within the range data for historical controls reported for the laboratory (Table 3).

+Recommended by Subdivision F (October 1982) Guidelines.

Table 2. Representative Mean Cholinesterase Activity of Rats Treated Dermally with Chlorpyrifos for 21 Days[a]

Dosage level (mg/kg/day)	Cholinesterase activity		
	Plasma (U/mL)	RBC (U/mL)	Brain (U/g)
	Males		
0	7.8 ± 0.5	13.8 ± 0.7	131.7 ± 3.2
0.1	7.6 ± 0.3	13.8 ± 0.6	133.1 ± 2.5
0.5	8.2 ± 0.8	14.0 ± 0.6	130.8 ± 4.4
1	7.4 ± 0.7	14.1 ± 0.6	129.5 ± 3.0
5	7.3 ± 0.5	12.3 ± 2.0	128.3 ± 4.5
	Females		
0	34.6 ± 4.3	14.0 ± 0.3	126.0 ± 19.8
0.1	40.2 ± 6.9	14.3 ± 0.8	135.0 ± 2.8
0.5	33.0 ± 5.7	13.8 ± 1.5	135.8 ± 3.3
1	38.2 ± 2.6	13.5 ± 0.5	135.5 ± 6.5
5	28.4 ± 5.6	13.9 ± 0.7	133.5 ± 5.3

[a]Based on five animals/sex/dose

Data was extracted from Tables 26 an 27, pages 52 and 53 of the report.

TABLE 3. Historical Control Cholinesterase Activity Data for Fischer 344 Rats[a]

	Cholinesterase activity		
	Plasma (U/mL)	RBC (U/mL)	Brain (U/g)
2-Week Inhalation Study			
Males			
Mean ± S.D	6.6 ± 0.7	12.2 ± 1.0	130.1 ± 4.7
Minimum	5.8	10.8	119.0
Maximum	8.0	14.2	135.6
Total No. animals	36	12	12
Females			
Mean ± S.D.	22.6 ± 3.1	12.4 ± 1.0	139.9 ± 3.6
Minimum	17.2	10.8	134.2
Maximum	29.5	14.8	145.3
Total No. animals	36	12	12
13-Week Inhalation Study			
Males			
Mean ± S.D.	7.6 ± 0.8	14.7 ± 0.8	132.0 ± 2.3
Minimum	6.7	13.2	128.6
Maximum	9.3	15.8	135.8
Total No. animals	10	10	10
Females			
Mean ± S.D.	31.6 ± 6.1	12.8 ± 1.6	133.9 ± 4.3
Minimum	22.9	11.2	123.7
Maximum	40.3	14.8	138.4
Total No. animals	9	9	9

[a]Data extracted from study report Tables 26 and 27, pages 52 and 53, MRID No. 409728-01. Dow Chemical Company.

6. Urinalysis: Urine was collected from fasted animals at study termination. The CHECKED (X) parameters were examined:

X	Appearance*	X	Glucose*
	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Results: There were no toxicologically important effects on urinary parameters.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In situ examinations of the eyes were performed by the glass slide technique. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	Aorta*	XX Brain*
Salivary glands*	X Heart	X Peripheral nerve (sciatic nerve)*
Esophagus*	Bone marrow*	X Spinal cord (3 levels)
Stomach*	Lymph nodes*	Pituitary*
Duodenum*	Spleen	Eyes (optic nerve)*
Jejunum*	Thymus	
Ileum*		
Cecum*		
Colon*		
Rectum	**Urogenital**	**Glandular**
XX Liver	XX Kidneys*	XX Adrenals*
Gallbladder*	Urinary bladder*	Lacrimal gland
Pancreas*	XX Testes*	Mammary gland
	Epididymides	Thyroids*
	Prostate	Parathyroids*
Respiratory	Seminal vesicle	Harderian glands
Trachea*	Ovaries	
Lung*	Uterus*	

Other
Bone (sternum)*
Skeletal muscle*
X Skin (treated and untreated sites
X All gross lesions and masses

Microscopic examinations were performed on control and high-dose animals only.

*Recommended by Subdivision F (October 1982) Guidelines.

Results:

a. Organ Weights: Organ weights of dosed males and females were similar to concurrent controls.

b. Gross Pathology: It was reported that no changes of toxicologic significance were evident in macroscopic pathology.

c. Microscopic Pathology:

Nonneoplastic: No compound-related indication of toxicity was observed in dosed animals. A few sporadic changes were considered to be incidental and were unrelated to treatment. One control female exhibited urethral and urinary bladder hyperplasia and inflammation.

D. STUDY AUTHORS' CONCLUSIONS:

When female Fischer 344 rats were exposed dermally to chlorpyrifos in corn oil at dosages of 0, 1, 10, 100, o4 500 mg/kg/day for 4 days, there were marked dose-related decreases in plasma cholinesterase activities at the three highest dosages. Erythrocyte cholinesterase activities were also decreased in a dose-related but less dramatic fashion. Cholinesterase activity of rats dosed with 1 mg/kg/day were within the range of historical controls. No other compound-related signs of toxicity were exhibited. Based on decreases in cholinesterase activity at 10 mg/kg/day, rats were subsequently exposed dermally to chlorpyrifos in corn oil at doses of 0, 0.1, 0.5, 1, or 5 mg/kg/day for 21 days. No compound-related signs of toxicity were observed at any dose level. Dermal application of 5 mg/kg/day over 21 days showed no evidence of systemic toxicity and no decrease in cholinesterase activity, although application of 10 mg/kg/day for 4 days produced decreases in plasma and erythrocyte cholinesterase activities.

E. REVIEWERS' discussion and interpretation of results:

The design and conduct of the study were acceptable; however, the following deficiencies were found. The 4-day probe study was conducted using only female rats; both sexes should have been included in the study for a complete observation. Quantitative results of the homogeneity, concentration, and stability analyses of the test compound were not included in the study report. The test material dosing solutions were analyzed prior to study initiation only. Total bilirubin was not measured, as recommended in clinical chemistry parameters of the EPA Pesticide Assessment Guideline, 1982, for 21-day dermal toxicity studies. These guidelines state that the highest dose level tested in a 21-day dermal toxicity study "should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation." No systemic or dermal compound-related effects were found at any dose

level of this study. This 21-day study will be considered acceptable from a "Toxic Effects" standpoint in view of the cholinesterase inhibition observed at 10 mg/kg/day in the 4-day dermal probe study.

This study is classified Core Supplementary. It may be upgraded if the registrant's response to the following is acceptable:

The registrant is requested to provide data concerning the results of homogeneity, concentration and stability of the test article dosing solutions.

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Chemical:	**Chlorpyrifos (ANSI)**
PC Code:	**059101**
HED File Code	**13000 Tox Reviews**
Memo Date:	**05/18/1989**
File ID:	**TX007188**
Accession Number:	**412-01-0126**

HED Records Reference Center
03/21/2001